REMARKS

In the Office Action dated November 1, 2006, claims 1 and 35-89 are pending. Claims 41-46, 52-53, 58-59, 64-68 and 73-89 are withdrawn from further consideration as directed to non-elected subject matter. Claims 1, 35-40, 47-51, 54-57, 60-63 and 69-72 are examined on the merits to the extent that these claims read on the elected species. The specification is objected to for certain alleged informalities. Claim 1 is objected to under 37 C.F.R. §1.175 as a substantial duplicate of claim 35. Claims 47-48, 50, 54, 56, 60, 62 and 69-72 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the written description requirement. Claims 1, 35-38, 40, 48-49, 54-55, 60-61 and 69-71 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998), allegedly as evidenced by the pPICZB vector diagram. Claims 1, 35-40 and 69 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Chiba et al. (*J. Biol. Chem.* 41: 26298-26304, 1998), as allegedly evidenced by Inoue et al. (*Biochim. Biophys. Acta* 1253: 141-145, 1995). Claims 47-49, 54-57, 60-61 and 69-71 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by JP 8-336387. Claims 1, 35-39, 47-51, 54-57, 60-63 and 69-72 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Gerngross (US 2002/0137134), allegedly as evidenced by JP 8-336387. Claims 48-51, 54-57, 60-63, 69-72 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Martinet et al. in view of JP 8336387. Claims 1, 35, 38, 48-51, 54-57, 60-63, 69 and 71-72 are rejected under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1-28 of U.S. patent No. 6,803,225. Claims 48-51 and 69-72 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 83, 88, 126-135 of co-pending Application No. 10/185,475.

This Response addresses each of the Examiner's rejections and objections. Specifically, Applicants have canceled claims 1 and 35-73 and have added claims 90-113 by way of the instant amendment.

New claims 90-104 are directed to a genetically engineered strain of *Pichia*, wherein the strain is transformed with a vector capable of expressing a *T.reesei* α-1,2-mannosidase or a functional part thereof, the vector comprising a nucleotide sequence coding for said α-1,2-mannosidase or said functional part, and wherein the genomic Och1 gene in the strain is disrupted such that the strain fails to produce a functional Och1 protein (see independent claim 90). Support for claims 90-104 is found throughout the specification and in previous claims 35-53, for example. Support for the feature that the strain produces $Man_5GlcNac_2$ is found in the specification page 45, lines 12-14.

New claim 105 is directed to a kit, which comprises a strain a defined in certain preceding claims. Support for claim 105 is found in the specification and previous claims 69-73, for example.

New claims 106-113 are directed to methods for producing a glycoprotein with reduced glycosylation by utilizing the strains of the present invention. Support for these claims is found in previous claims 54-65, for example.

Applicants respectfully submit that the foregoing amendment does not introduce new matter and places the present application is in condition for allowance. Favorable consideration of all pending claims is therefore respectfully requested.

Turning to the specific rejections and objections raised in the Office Action, the Examiner has objected to the specification for containing an embedded hyperlink on page 40,

line 19 and page 44, line 14. In addition, the Examiner indicates that the peptide sequence HDEL has not been identified with its proper SEQ ID NO: 1, on page 4, lines 4 and 17.

In response, Applicants have amended the specification to delete the hyperlinks, and to insert "SEQ ID NO: 1" where appropriate. In view of the foregoing amendment, the objection to the specification is overcome and withdrawal thereof is respectfully requested.

Claim 1 is objected to under 37 C.F.R. §1.175 as a substantial duplicate of claim 35.

It is respectfully submitted that the objection is rendered moot in view of the cancellation of claims 1 and 35. Withdrawal of the objection is therefore respectfully requested.

Claims 47-48, 50, 54, 56, 60, 62 and 69-72 are rejected under 35 U.S.C. §112, first paragraph, as allegedly failing to satisfy the written description requirement. Specifically, the Examiner contends that the specification fails to provide sufficient written description for a representative number of OCH1 genes derived from a broad genus of methylotrophic yeasts, including *Candida*, *Hansenuala*, *Torulopsi* and *Pichia*, to be used in a vector, a kit, and the related methods, as claimed. According to the Examiner, little is known about the existence and/or structures of OCH1 genes from other genera of methylotrophic yeasts such as *Candida*, *Hansenuala* and *Torulopsis*.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant rejection moot. The new claims recite specifically a strain of *Pichia*, wherein the genomic Och1 gene in the strain is disrupted such that the strain fails to produce a functional Och1 protein. It is respectfully submitted that the claims, as presently recited, are fully supported by the instant disclosure, especially the example (pages 44-45) wherein the genomic Och1 gene in a *Pichia pastoris* strain has been disrupted. As such, it is respectfully submitted that the subject matter as presently claimed fully complies with the written description requirement under

35 U.S.C. §112, first paragraph. Withdrawal of the written description rejection is respectfully requested.

Claims 1, 35-38, 40, 48-49, 54-55, 60-61 and 69-71 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Martinet et al. (*Biotechnology Letters* 20: 1171-1177, 1998), allegedly as evidenced by the pPICZB vector diagram. Specifically, the Examiner contends that Martinet et al. teach the preparation of plasmids for expression of *T. reesei* α-1,2-mannosidase in *Pichia pastoris*.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant rejection moot. The new claims recite specifically a strain of *Pichia*, wherein the strain is transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof, and wherein the genomic Och1 gene in the strain is disrupted such that the strain fails to produce a functional Och1 protein. Applicants respectfully submit that Martinet et al. do not teach anywhere disrupting the genomic Och1 gene in a strain of *Pichia* that is transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase. Accordingly, Martinet et al. do not teach the invention as presently claimed. Withdrawal of the rejection under 35 U.S.C. §102(b) based on Martinet et al. is respectfully requested.

Claims 1, 35-40 and 69 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by Chiba et al. (*J. Biol. Chem.* 41: 26298-26304, 1998), as allegedly evidenced by Inoue et al. (*Biochim. Biophys. Acta* 1253: 141-145, 1995). According to the Examiner, Chiba et al. teach the preparation of an expression vector encoding HDEL-tagged *Aspergillus* α-1, 2-mannosidase for expression in various *Saccharomyces cerevisiae* strains. In addition, the Examiner states that Chiba et al. disclose that the Δoch1 mnn1 double mutant yeasts are useful for the production of recombinant therapeutic glycoproteins without any antigenicity toward humans, which is due to

the accumulation of a single oligosaccharide moiety $Man_8GlcNAc_2$ in the double mutant instead of the highly antigenic mature mannan glycans formed in wild-type yeast cells.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant rejection moot. The new claims recite specifically a strain of *Pichia*, wherein the strain is transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof, and wherein the genomic Och1 gene in the strain is disrupted such that the strain fails to produce a functional Och1 protein. Chiba et al. do not teach any genetically engineered *Pichia* strain, or a strain capable of expressing a *T. reesei* α-1,2-mannosidase, or a strain having its genomic Och1 gene disrupted such that the strain fails to produce a functional Och1 protein.

In this regard, Applicants submit that a rejection of a claim under 35 U.S.C. §102(b) requires that the single prior art reference disclose every element of the claim. The absence from the reference of any claimed element negates anticipation. Kloster Speedsteel AB v Crucible Inc., 793 F.2d 1565, 1571, 230 USPQ 81, 84 (Fed. Cir. 1986). In the present case, Chiba et al. simply do not teach each and every element of the presently claimed invention. Accordingly, withdrawal of the rejection under 35 U.S.C. §102(b) based on Chiba et al. is respectfully requested.

Claims 47-49, 54-57, 60-61 and 69-71 are rejected under 35 U.S.C. §102(b) as allegedly anticipated by JP 8-336387. The Examiner states that JP 8-336387 teaches a vector construct comprising a portion of *Pichia* OCH1 gene and a selectable marker gene for disruption of the genomic OCH1 in a *Pichia* yeast strain for inhibiting the elongation of sugar chains on glycoproteins.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant rejection moot. The new claims recite a strain of *Pichia*, wherein the strain is transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof, in addition to having the genomic Och1 gene disrupted. The reference does not teach anywhere the introduction of a vector into a *Pichia* strain for expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof. Therefore, JP 8-336387 simply does not teach the invention as presently recited. Withdrawal of the rejection under 35 U.S.C. §102(b) based on JP 8-336387 is respectfully requested.

Claims 1, 35-39, 47-51, 54-57, 60-63 and 69-72 are rejected under 35 U.S.C. §102(e) as allegedly anticipated by Gerngross (US 2002/0137134), allegedly as evidenced by JP 8-336387.

According to the Examiner, Gerngross discloses methods by which fungi including *Pichia pastoris* can be genetically modified to produce glycosylated proteins having patterns of glycosylation similar to that of animal cells. Specifically, Gerngross allegedly teaches a microorganism that is engineered to express an exogenous α-1, 2-mannosidase (including α-1, 2-mannosidase from *Trichoderma reesei*, paragraph 0036) having an optimal pH between 5.1 and 8.0, wherein the enzyme is targeted to the endoplasmic reticulum (ER) or Golgi apparatus of the host organism, where the host trims N-glycans such as $Man_8GlcNAc_2$ to yield $Man_5GlcNAc_2$. Gerngross also allegedly teaches that mutant strains that do not express one or more enzymes involved in the production of high mannose structures can be used, and that such mutant strains can be used in conjunction with the introduction of an α-1, 2-mannosidase. Gerngross further allegedly refers to JP 8-336387 for a hypermannosylation-minus (OCH1) mutant *of Pichia pastoris* in this context.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant rejection moot. Applicants further respectfully submit that Gerngross does not teach the strains and methods, as presently claimed. Specifically, Gerngross teaches that *Pichia pastoris* can be genetically modified for the purpose disclosed therein; and in a separate context, Gerngross teaches that an exogenous α-1, 2-mannosidase (including α-1, 2-mannosidase from *Trichoderma reesei*) can be introduced into a microorganism. However, Gerngross does not teach a genetically engineered strain of *Pichia* transformed with a vector capable of expressing a *T. reesei* α-1,2-mannosidase or a functional part thereof, as presently claimed. In fact, Gerngross teaches away from such a strain. Specifically, Gerngross discloses that to obtain $Man_5GlcNAc_2$ in high yield, one could engineer a strain that expresses a α-1,2 mannosidase, which should have an optimal pH that is between 5.1 and 8.0. According to Gerngross, the enzymes having an optimal pH of 5.0 would most likely provide insufficient activity in the ER or early Golgi. See paragraph 0068 of Gerngross, for example. Applicants observe that the *T. reesei* α-1,2-mannosidase has an optimal pH of 5.0, according to Table 3 of Gerngross. Gerngross also references the *T. reesei* α-1,2-mannosidase in several passages that discuss the inefficiency of this enzyme. See paragraph 0068 and paragraph 0071 of Gerngross, for example.

Moreover, with respect to the teaching of Gerngross relating to Och1, Gerngross merely generally discloses reducing endogenous mannosyltransferase activity. In this regard, Gerngross lists several enzymes involved in hypermannosylation, including OCH1, MNN4, MNN6, and MNN1. See Table 6. It is not clear, based on the disclosure of Gerngross, as to whether the disruption of OCH1 alone in a *Pichia* strain would be sufficient, or whether multiple enzymes need to be disrupted, in order to obtain Man_8 and ultimately Man_5 N-glycans.

Further, although Gerngross refers to JP 8-336387 for an Och1 mutant strain of *P. pastoris*, this Japanese application does not appear to provide any showing that the predominant N-glycan form in the mutant is a Man_8 glycan (which is believed to ultimately give rise to Man_5 N-glycans). In fact, a recent report published by Glycofi (assignee of the Gerngross publication) shows that Man_9 is still a predominant N-glycan form in the Och1 mutant. See Choi et al., *Proc. Natl. Acad. Sci.* 100: 5022-5027 (2003) (attached as **Exhibit 1**), Figure 3B.

Accordingly, Applicants respectfully submit that Gerngross merely provides numerous potential options for those skilled in the art to experiment, and does not provide clear teaching that anticipates the invention as presently claimed. Accordingly, the rejection under 35 U.S.C. §102(b) based on Gerngross is overcome and withdrawal thereof is respectfully requested.

Claims 48-51, 54-57, 60-63 and 69-72 are rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Martinet et al. in view of JP 8336387.

According to the Examiner, Martinet et al. also discloses expression of *T. reesei* α-1, 2-mannosidase in a *Pichia pastoris* strain, together with a heterologous glycoprotein. The co-expression of the chimeric MNS1/*T. reesei* α-1, 2-mannosidase resulted in the formation of both trimmed and hyperglycosylation glycan products of hemagglutinin (HA). The Examiner refers to page 1176, col. 1 of Martinet et al., where the authors note that hyperglycosylation can be prevented by expressing a protein of interest in the mutant yeast strains mnn9, och1 or in the temperature-sensitive strain ngd-29, where N-glycosylation is confined to the core oligosaccharide residues. The Examiner acknowledges that Martinet et al. do not teach to further transform the *Pichia pastoris* strain with a vector to effect the disruption of the genomic Och1 gene in the *Pichia pastoris* strain in order to reduce glycosylation. However, the Examiner

contends that at the effective filing date of the present application, JP 8-336387 already taught the preparation of a vector construct for disruption of the genomic OCH1 in a *Pichia* yeast strain. The Examiner concludes that the references in combination have provided a motivation and a reasonable expectation of success for those skilled in the art to combine the respective teachings of the references and arrive at the claimed invention.

Applicants respectfully disagree with the Examiner. Although Martinet et al. refer to the use of mutant yeast strains mnn9, och1 and ngd-29, where N-glycosylation is confined to the core oligosaccharide residues, Applicants respectfully submit that the mutant OCH1 strain is a *S. cerevisiae* strain, as evidenced by the corresponding literature publication cited by Martinet et al. at page 1176, left column (abstract attached as **Exhibit 2**). It was known that various fungal species employ different glycosylation enzymes and differ in their glycosylation patterns, as explicitly acknowledged by Martinet et al. See, e.g., page 1171, right column, middle paragraph; and page 1176, left column. In fact, in the immediate context of discussing the use of the *S. cerevisiae* mutants for preventing hyperglycosylation, Martinet et al. state that "the glycosylation pathways of *S. cerevisiae* and *P. pastoris* are significantly different".

Moreover, as discussed above in connection with Choi et al. (**Exhibit 1**), Man_9 is still a predominant N-glycan form in the Och1 mutant of *Pichia pastoris* disclosed in Choi et al. Those skilled in the art would have expected to produce the Man_5 glycan structure from a Man_8 glycan, not from Man_9. Therefore, Applicants respectfully submit that those skilled in the art would not have considered it obvious, simply based on the disclosures of Martinet et al. and JP 8336387, to obtain a *Pichia pastoris* strain, as presently claimed, that is capable of producing the desired Man_5 glycan structure. In this regard, Applicants have added claims 96 and 113 to

further delineate the feature of the instant strain, directed to the production of the Man_5 glycan structure.

In view of the foregoing, Applicants respectfully submit that the subject matter as presently claimed is not obvious in view of Martinet et al. and JP 8336387. Withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

Claims 1, 35, 38, 48-51, 54-57, 60-63, 69 and 71-72 are rejected under the judicially created doctrine of obviousness-type double patenting as allegedly unpatentable over claims 1-28 of U.S. patent No. 6,803,225. Claims 48-51 and 69-72 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 83, 88, 126-135 of co-pending Application No. 10/185,475.

Applicants respectfully submit that the rejected claims have been canceled, which renders the instant obviousness-type double patenting rejections moot. In the event that the Examiner finds the rejections apply to the newly presented claims, Applicants intend to file a terminal disclaimer to obviate such rejections.

In view of the foregoing amendments and remarks, it is firmly believed that the subject application is in condition for allowance, which action is earnestly solicited.

Respectfully submitted,

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